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FILE 'HOME' ENTERED AT 10:41:26 ON 23 MAR 2005

=> s (adenylyl cyclase or adenylate cyclase)
THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
Some commands only work in certain files. For example, the EXPAND command can only be used to look at the index in a file which has an index. Enter "HELP COMMANDS" at an arrow prompt (=>) for a list of commands which can be used in this file.

=> file caplus
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.42	0.42

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FILE COVERS 1907 - 23 Mar 2005 VOL 142 ISS 13
FILE LAST UPDATED: 22 Mar 2005 (20050322/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> s (adenylyl cyclase or adenylate cyclase)
8750 ADENYLYL
46756 CYCLASE
1997 CYCLASES
47018 CYCLASE
(CYCLASE OR CYCLASES)
7235 ADENYLYL CYCLASE
(ADENYLYL(W)CYCLASE)
37364 ADENYLATE
695 ADENYLATES
37691 ADENYLATE
(ADENYLATE OR ADENYLATES)
46756 CYCLASE
1997 CYCLASES
47018 CYCLASE
(CYCLASE OR CYCLASES)
30284 ADENYLATE CYCLASE
(ADENYLATE(W)CYCLASE)
L1 35361 (ADENYLYL CYCLASE OR ADENYLATE CYCLASE)

=> s (guanylyl(W)cyclase or guanylate(W) cyclase)
3764 GUANYLYL
46756 CYCLASE

```
          1997 CYCLASES
         47018 CYCLASE
                 (CYCLASE OR CYCLASES)
          2478 GUANYLYL(W)CYCLASE
          7172 GUANYLATE
            70 GUANYLATES
          7205 GUANYLATE
                 (GUANYLATE OR GUANYLATES)
         46756 CYCLASE
          1997 CYCLASES
         47018 CYCLASE
                 (CYCLASE OR CYCLASES)
          5752 GUANYLATE(W) CYCLASE
L2        8006 (GUANYLYL(W)CYCLASE OR GUANYLATE(W) CYCLASE)

=> s L1 and L2
L3         873 L1 AND L2

=> s (fluorecent detection)
             70 FLUORECENT
         553435 DETECTION
           2843 DETECTIONS
         555111 DETECTION
                 (DETECTION OR DETECTIONS)
L4             1 (FLUORECENT DETECTION)
                 (FLUORECENT(W)DETECTION)

=> s ( fluorescent detection)
         136429 FLUORESCENT
             38 FLUORESCENTS
         136440 FLUORESCENT
                 (FLUORESCENT OR FLUORESCENTS)
         553435 DETECTION
           2843 DETECTIONS
         555111 DETECTION
                 (DETECTION OR DETECTIONS)
L5           871 ( FLUORESCENT DETECTION)
                 (FLUORESCENT(W)DETECTION)

=> L3 and L4 and L5
L3 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s L3 and L4 and L5
L6             0 L3 AND L4 AND L5

=> s L3 and L5
L7             0 L3 AND L5

=> s L1 AND L2
L8           873 L1 AND L2

=> s L8 AND L5
L9             0 L8 AND L5

=> s (fluorescent nucleotide)
         136429 FLUORESCENT
             38 FLUORESCENTS
         136440 FLUORESCENT
                 (FLUORESCENT OR FLUORESCENTS)
         374142 NUCLEOTIDE
         109330 NUCLEOTIDES
         426549 NUCLEOTIDE
                 (NUCLEOTIDE OR NUCLEOTIDES)
L10          195 (FLUORESCENT NUCLEOTIDE)
                 (FLUORESCENT(W)NUCLEOTIDE)
```

```
=> s L10 AND detection
        553435 DETECTION
          2843 DETECTIONS
        555111 DETECTION
                  (DETECTION OR DETECTIONS)
L11          42 L10 AND DETECTION

=> s Cyclase AND L11
         46756 CYCLASE
          1997 CYCLASES
         47018 CYCLASE
                  (CYCLASE OR CYCLASES)
L12           0 CYCLASE AND L11

=> s L1 AND L10
L13           0 L1 AND L10

=> s BODIPY
L14         948 BODIPY

=> s GTP and L14
         34187 GTP
            75 GTPS
         34216 GTP
                  (GTP OR GTPS)
L15          29 GTP AND L14

=> d ti so abs 1-10 L15

L15  ANSWER 1 OF 29  CAPLUS  COPYRIGHT 2005 ACS on STN
TI   Real-time Detection of Basal and Stimulated G Protein GTPase Activity
     Using Fluorescent GTP Analogues
SO   Journal of Biological Chemistry (2005), 280(9), 7712-7719
     CODEN: JBCHA3; ISSN: 0021-9258
AB   Hydrolysis of fluorescent GTP analogs BODIPY FL
     guanosine 5'-O-(thiotriphosphate) (BGTPγS) and  BODIPY FL
     GTP (BGTP) by Gαi1 and Gα was characterized using
     online capillary electrophoresis o laser-induced fluorescence assays in
     order that changes in substrate, substrate-enzyme complex, and product
     could be monitored sep.  Apparent k values (V /[E]) max cat steady-state
     and Km values were determined from assays for each substrate-protein pair.
     When BGTP was the substrate, maximum turnover nos. for Gα and
     Gαi1 were 8.3 ± 1 x 10-3 and 3.0 ± 0.2 x 10-2 s-1, resp., and
     Km values were 120 ± 60 and 940 ± 160 nM.  Assays with BGTPγS
     yielded maximum turnover nos. of 1.6 ± 0.1 x 10-4 and 5.5 ± 0.3 x 10-4
     s-1 for Gα and Gαi1; Km values were 14 o±8 and 87 ± 22
     nM.  Acceleration of Gα GTPase activity by regulators of G protein
     signaling (RGS) was demonstrated in both steady-state and
     pseudo-single-turnover assay formats with BGTP.  Nanomolar RGS increased
     the rate of enzyme product formation (BODIPY FL GDP (BGDP)) by
     117-213% under steady-state conditions and accelerated the rate of G
     protein-BGTP complex decay by 199-778% in pseudo-single-turnover assays.
     Stimulation of GTPase activity by RGS proteins was inhibited 38-81% by 40
     μM YJ34, a previously reported peptide RGS inhibitor.  Taken together,
     these results illustrate that Gα subunits utilize BGTP as a
     substrate similarly to GTP, making BGTP a useful fluorescent
     indicator of G protein activity.  The unexpected levels of BGTPγS
     hydrolysis detected suggest that caution should be used when interpreting
     data from fluorescence assays with this probe.

L15  ANSWER 2 OF 29  CAPLUS  COPYRIGHT 2005 ACS on STN
TI   Palmitoylation regulates GDP/GTP exchange of G protein by
     affecting the GTP-binding activity of Goα
SO   International Journal of Biochemistry & Cell Biology (2005), 37(3),
     637-644
     CODEN: IJBBFU; ISSN: 1357-2725
AB   The effect of palmitoylation on the GTP-binding activity and
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conformation of Goα protein in hydrophobic and hydrophilic environments was studied. The binding assay was performed with an isotope labeled analog of **GTP**, **GTP**-γ-35S, and its fluorescent analog, **BODIPY** FL-**GTP**γS was used to detect conformational change in the **GTP**-binding domain of Goα. Investigation of the **GTP**-γ-35S binding activity of Goα shows that in a hydrophobic environment, mimicked by the presence of detergent, the apparent dissociation constant for palmitoylated Goα (KD = 25.5 + 10-9 ± 1.7 + 10-9 M) increased threefold compared with that of non-palmitoylated Goα (KD = 9.9 + 10-9 ± 0.8 + 10-9 M), while in an aqueous environment without detergent there is no significant difference between palmitoylated (KD = 50.1 + 10-9 ± 5.2 + 10-9 M) and non-palmitoylated (KD = 65.5 + 10-9 ± 7.6 + 10-9 M) Go(. This indicates that in a membrane environment palmitoylation may weaken the **GTP**γS binding ability of Go(. Fluorescent quenching studies using **BODIPY** FL-**GTP**γS as a probe showed that the conformation of the **GTP**-binding domain of Go(tends to become more compact after palmitoylation. These results imply that palmitoylation may regulate the **GTP**/GDP exchange of Goα by influencing the **GTP** -binding activity of Goα and facilitating the on-off switch function of the G protein in G protein-coupled signal transduction.

L15 ANSWER 3 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Transcriptional incorporation of adenine analogs into RNA and use of the analog-containing RNAs
SO U.S. Pat. Appl. Publ., 25 pp.
CODEN: USXXCO
AB Methods of incorporating adenosine analogs and derivs. into the 5'-ends of an RNA by transcription are described. These adenosine derivs. may include naturally occurring compds. such as CoA, NAD, and FAD, as well as synthetic analogs containing reactive groups or nuclease-resistant phosphate backbone analogs. The derivs. can be used to impart desirable properties to the RNA such as fluorescence, the ability to bind to receptors or ligands, and improved catalytic activity. The transcribed RNAs can be used in a variety of applications including nucleic acid detection, designed or random generation of catalytic RNAs, antisense applications, and in the study of RNA structure and function. The incorporation is achieved by in vitro transcription using all four nucleoside triphosphates and the triphosphate of the adenine analog. The analog is present at significantly higher concentration than the ATP.

L15 ANSWER 4 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI High throughput screening fluorescent assay for guanylyl and adenylyl cyclase
SO U.S. Pat. Appl. Publ., 14 pp.
CODEN: USXXCO
AB The present invention relates to methods of assaying nucleotide cyclase activity. In particular, the present invention relates to fluorescence-based methods of assaying guanylyl and adenylyl cyclase activity using fluorescently labeled **GTP**γS. The assay can be used for high throughput screening of both activators and inhibitors of cyclase.

L15 ANSWER 5 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Affinity Assays Using Fluorescence Anisotropy with Capillary Electrophoresis Separation
SO Analytical Chemistry (2004), 76(24), 7380-7386
CODEN: ANCHAM; ISSN: 0003-2700
AB A novel approach to detecting affinity interactions that combines fluorescence anisotropy with capillary electrophoresis (FACE) was developed. In the method, sample is injected into a capillary filled with buffer that contains a fluorescent probe that possesses low fluorescence anisotropy. If proteins or other large mols. in the sample bind the fluorescent probe, their migration through the capillary can be detected as a pos. anisotropy shift. Thus, the method provides both separation and confirmation of binding to the probe. Calcns. based on combining the Perrin equation and dissociation constant were used to predict the effect of

conditions on aniostropy detection. These calcns. predict that low probe concns. yield the best sensitivity while higher concns. increase the dynamic range for detection of binding partner. The assay was applied to detection of G proteins using **BODIPY** FL **GTP**γS as the fluorescent probe. Exptl. measurements exhibited trends in anisotropy with varying probe and protein concns. that were consistent with the calcns. The limit of detection for Gαi1 was 3 nM when the electrophoresis buffer contained 250 nM **BODIPY** FL **GTP** γS. FACE affinity assay is envisioned as a method that can quantify selected binding partners and screen complex samples for compds. that possess affinity for a particular small mol. that is used as a probe.

L15 ANSWER 6 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Use of a native affinity ligand for the detection of G proteins by capillary isoelectric focusing with laser-induced fluorescence detection
SO Electrophoresis (2004), 25(14), 2319-2325
CODEN: ELCTDN; ISSN: 0173-0835
AB Affinity probe capillary isoelec. focusing (CIEF) with laser-induced fluorescence was explored for detection of Ras-like G proteins. In the assay, a fluorescent **BODIPY** FL **GTP** analog (BGTPγS) and G protein were incubated resulting in formation of BGTPγS-G protein complex. Excess BGTPγS was separated from BGTPγS-G protein complex by CIEF using a 3-10 pH gradient and detected in whole-column imaging mode. In other cases, a single point detector was used to detect zones during the focusing step of CIEF using a 2.5-5 pH gradient. In this case, analyte peaks passed the detector in .apprx. 5 min at an elec. field of 350 V/cm. Detection during focusing allowed for more reproducible assays at shorter times but with a sacrifice in sensitivity compared to detection during mobilization. Resolution was adequate to sep. BGTPγS-Ras and BGTPγS-Rab3A complexes. Formation of specific complexes was confirmed by adding **GTP** γS to samples containing BGTPγS-G protein. **GTP**γS competed with BGTPγS for G protein binding sites resulting in decreased BGTPγS-G protein peak heights. The concentrating effect of CIEF enabled detection limits of 30 pM.

L15 ANSWER 7 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Stabilization of an Intermediate Activation State for Transducin by a Fluorescent **GTP** Analogue
SO Biochemistry (2004), 43(27), 8778-8786
CODEN: BICHAW; ISSN: 0006-2960
AB The **GTP**-binding protein (G protein), transducin, serves as a key mol. switch in vertebrate vision through the tight regulation of its **GTP**-binding (activation)/**GTP** hydrolytic (deactivation) cycle by the photoreceptor rhodopsin. To better understand the structure-function characteristics of transducin activation, we have set out to identify spectroscopic probes that bind to the guanine nucleotide-binding site of this G protein and maintain its ability to interact with its specific cellular target/effector, the cyclic GMP phosphodiesterase (PDE). In this study, we describe the characterization of a fluorescently labeled **GTP** analog, **BODIPY**-FL **GTP**γS (BOD- **GTP**γS), that binds to the alpha subunit of transducin (αT) in a rhodopsin- and Gβγ-dependent manner, similar to the binding of **GTP** or **GTP** γS, with an apparent dissociation constant of 100 nM. The rhodopsin-dependent binding of BOD-**GTP**γS to αT is slow, relative to the rate of binding of **GTP**γS, particularly under conditions where rhodopsin must act catalytically to stimulate the exchange of BOD-**GTP**γS for GDP on multiple αT subunits. This reflects a slower rate of dissociation of rhodopsin and Gβγ from αT-BOD- **GTP**γS complexes, relative to their rates of dissociation from αT- **GTP**γS. The binding of BOD-**GTP**γS occurs without a change in the intrinsic tryptophan fluorescence of αT, indicating that only a subtle movement of the Switch 2 domain on αT accompanies the binding of this **GTP**γS analog. Nevertheless, the BOD- **GTP** γS-bound αT subunit is able to bind with high affinity to the recombinant, purified γ subunit of PDE (γPDE) labeled with

5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (IAEDANS (Kd ≈ 13 nM)), as well as bind to and stimulate the activity of PDE, albeit less efficiently compared to αT- **GTP**γS. Taken together, these findings suggest that the binding of BOD-**GTP** γS to transducin causes it to adopt a distinct conformation that appears to be intermediate between the inactive and fully active states of αT, and this fluorescent nucleotide analog can be used as a reporter group to characterize the interactions of αT in this conformational state with its biol. target/effector.

L15 ANSWER 8 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Spontaneous nucleotide exchange in low molecular weight GTPases by fluorescently labeled γ-phosphate-linked **GTP** analogs. [Erratum to document cited in CA140:419768]
SO Proceedings of the National Academy of Sciences of the United States of America (2004), 101(14), 5180
CODEN: PNASA6; ISSN: 0027-8424
AB In the second to last sentence in the legend for Figure 7, the word "deleted" should read "detected" and the "6" of "His6" should be a subscript. The corrected figure and its legend are given.

L15 ANSWER 9 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Spontaneous nucleotide exchange in low molecular weight GTPases by fluorescently labeled γ-phosphate-linked **GTP** analogs
SO Proceedings of the National Academy of Sciences of the United States of America (2004), 101(9), 2800-2805
CODEN: PNASA6; ISSN: 0027-8424
AB Regulated guanosine nucleotide exchange and hydrolysis constitute the fundamental activities of low mol. weight GTPases. The authors show that three **GTP** analogs with **BODIPY** fluorophores coupled via the gamma phosphate bind to the GTPases Cdc42, Rac1, RhoA, and Ras and displace GDP with high intrinsic exchange rates in the presence of Mg2+ ions, thereby acting as synthetic, low mol. weight guanine nucleotide exchange factors. The accompanying large fluorescence enhancements (as high as 12-fold), caused by a reduction in guanine quenching of the environmentally sensitive **BODIPY** dye fluorescence on protein binding, allow for real-time monitoring of this spontaneous nucleotide exchange in the visible spectrum with high signal-to-noise ratios. Binding affinities increased with longer aliphatic linkers connecting the nucleotide and **BODIPY** fluorophore and were in the 10-100 nM range. Steady-state and time-resolved fluorescence spectroscopy showed an inverse relationship between linker length and fluorescence enhancement factors and differences in protein-bound fluorophore mobilities, providing optimization criteria for future applications of such compds. as efficient elicitors and reporters of nucleotide exchange. EDTA markedly enhanced nucleotide exchange, enabling rapid loading of GTPases with these probes. Differences in active site geometries, in the absence of Mg2+, caused qual. different reporting of the bound state by the different analogs. The **BODIPY** analogs also prevented the interaction of Cdc42 with p21 activated kinase. Together, these results validate the use of these analogs as valuable tools for studying GTPase functions and for developing potent synthetic nucleotide exchange factors for this important class of signaling mols.

L15 ANSWER 10 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Thermodynamic Characterization of the Binding of Activator of G Protein Signaling 3 (AGS3) and Peptides Derived from AGS3 with Gαi1
SO Journal of Biological Chemistry (2003), 278(51), 51825-51832
CODEN: JBCHA3; ISSN: 0021-9258
AB Activator of G protein signaling 3 (AGS3) is a guanine nucleotide dissociation inhibitor (GDI) that contains four G protein regulatory (GPR) or GoLoco motifs in its C-terminal domain. The entire C-terminal domain (AGS3-C) as well as certain peptides corresponding to individual GPR motifs of AGS3 bound to Gαi1 and inhibited the binding of **GTP** by stabilizing the GDP-bound conformation of Gαi1. The stoichiometry, free energy, enthalpy, and dissociation constant for binding of AGS3-C to Gαi1 were determined using isothermal titration calorimetry. AGS3-C possesses two apparent high affinity (Kd .apprx. 20 nM) and two apparent

low affinity (Kd .apprx. 300 nM) binding sites for Gαi1. Upon deletion of the C-terminal GPR motif from AGS3-C, the remaining sites were approx. equivalent with respect to their affinity (Kd .apprx. 400 nM) for Gαi1. Peptides corresponding to each of the four GPR motifs of AGS3 (referred to as GPR1, GPR2, GPR3, and GPR4, resp., going from N to C terminus) bound to Gαi1 with Kd values in the range of 1-8 μM. Although GPR1, GPR2, and GPR4 inhibited the binding of the fluorescent **GTP** analog **BODIPY**-FL-guanosine 5'-3-O-(thio)triphosphate to Gαi1, GPR3 did not. However, addition of N- and C-terminal flanking residues to the GPR3 GoLoco core increased its affinity for Gαi1 and conferred GDI activity similar to that of AGS3-C itself. Similar increases were observed for extended GPR2 and extended GPR1 peptides. Thus, while the tertiary structure of AGS3 may affect the affinity and activity of the GPR motifs contained within its sequence, residues outside of the GPR motifs strongly potentiate their binding and GDI activity toward Gαi1 even though the amino acid sequences of these residues are not conserved among the GPR repeats.

=> d ti, so, abs 11-20 L15

L15 ANSWER 11 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Low-affinity interactions of **BODIPY**-FL-**GTP**γS and **BODIPY**-FL-GppNHp with Gi- and Gs-proteins
SO Naunyn-Schmiedeberg's Archives of Pharmacology (2003), 368(3), 210-215
CODEN: NSAPCC; ISSN: 0028-1298
AB **BODIPY**-FL-guanosine 5'-[γ-thio]triphosphate (B-**GTP** γS) and **BODIPY**-FL-guanosine 5'-[β,γ-imido]triphosphate (B-GppNHp) induce fluorescence changes upon binding to purified Gs/Gi-proteins and were suggested to serve as probes for monitoring receptor-mediated G-protein activation. However, B-**GTP** γS and B-GppNHp bound to receptor-Gαs/Gαi fusion proteins expressed in Sf9 cell membranes with 1,100- to 5,600-fold- and 17- to 55-fold lower affinity than **GTP**γS and GppNHp, resp. The affinity of B-**GTP**γS/B-GppNHp for Gs/Gi-proteins was considerably lower than the affinity of N-methylanthraniloyl (MANT)-substituted **GTP** analogs for Gs/Gi-proteins. B-**GTP**γS/B-GppNHp were much less potent than **GTP** γS/GppNHp at regulating adenylyl cyclase (AC) via Gs- and Gi-proteins. B-**GTP**γS/B-GppNHp were similarly efficient as **GTP**γS/GppNHp at activating Gi, but less efficient at activating Gs. In contrast to MANT-**GTP**γS/MANT-GppNHp, B-**GTP**γS/B-GppNHp were inefficient at directly inhibiting AC. In conclusion, the bulky **BODIPY** group strongly reduces the affinity of **GTP**γS/GppNHp for G-proteins, limiting the use of B-**GTP**γS/B-GppNHp as fluorescence probes.

L15 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Detection of G proteins by affinity probe capillary electrophoresis using a fluorescently labeled **GTP** analogue
SO Analytical Chemistry (2003), 75(16), 4297-4304
CODEN: ANCHAM; ISSN: 0003-2700
AB An affinity probe capillary electrophoresis (APCE) assay for guanine-nucleotide-binding proteins (G proteins) was developed using **BODIPY** FL **GTP**γS (BGTPγS), a fluorescently labeled **GTP** analog, as the affinity probe. In the assay, BGTPγS was incubated with samples containing G proteins and the resulting mixts. of BGTPγS-G protein complexes and free BGTPγS were separated by capillary electrophoresis and detected with laser-induced fluorescence detection. Sepns. were completed in less than 30 s using 25 mM Tris, 192 mM glycine at pH 8.5 as the electrophoresis buffer and applying 555 V/cm over a 4-cm separation distance. BGTPγS-Gαo peak heights increased linearly with Gαo up to .apprx.200 nM using a 50 nM BGTPγS probe. The detection limit for Gαo was 2 nM, corresponding to a mass detection limit of 3 amol. The high speed of the APCE assays allowed reaction kinetics and the dissociation constant (Kd) to be determined The on-rate and off-rate of BGTPγS to Gαo were 0.0068±0.0004 and 0.000 23±0.000 01 s-1, resp. The half-life of the

BGTPγS-Gαo complex was 3060±240 s and Kd was 8.6±0.7 nM. The ests. of these parameters are in good agreement with those obtained using established techniques, indicating the suitability of this method for such measurements. Lowering the temperature of the separation improved the detection of the complex, allowing the assay to be performed on a com. instrument with longer separation times. Addnl., the capability of the technique to detect several G proteins based on their binding to BGTPγS was demonstrated with assays for Gα and Gαi1 and for Ras and Rab3A.

L15 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Arrays of biological membranes and methods and use thereof
SO U.S. Pat. Appl. Publ., 35 pp., Cont.-in-part of U.S. Ser. No. 854,786.
CODEN: USXXCO
AB The present invention overcomes the problems and disadvantages associated with prior art arrays by providing an array comprising a plurality of biol. membrane microspots associated with a surface of a substrate that can be produced, used and stored, not in an aqueous environment, but in an environment exposed to air under ambient or controlled humidities. Preferably, the biol. membrane microspots comprise a membrane bound protein. Most preferably, the membrane bound protein is a G-protein coupled receptor, an ion channel, a receptor serine/threonine kinase or a receptor tyrosine kinase.

L15 ANSWER 14 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Labeled peptides, proteins and antibodies and processes and intermediates useful for their preparation
SO U.S. Pat. Appl. Publ., 54 pp., Cont.-in-part of Appl. No. PCT/US2000/26821.
CODEN: USXXCO
AB The invention provides peptide synthons having protected functional groups for attachment of desired moieties (e.g. functional mols. or probes). Also provided are peptide conjugates prepared from such synthons, and synthon and conjugate preparation methods including procedures for identifying the optimum probe attachment site. Biosensors are provided having environmentally sensitive dyes that can locate specific biomols. within living cells and detect chemical and physiol. changes in those biomols. as the living cell is moving, metabolizing and reacting to its environment. Methods are included for detecting **GTP** activation of a Rho GTPase protein using polypeptide biosensors. When the biosensor binds **GTP**-activated Rho GTPase protein, the environmentally sensitive dye emits a signal of a different lifetime, intensity or wavelength than when not bound. New fluorophores whose fluorescence responds to environmental changes are also provided that have improved detection and attachment properties, and that can be used in living cells, or in vitro.

L15 ANSWER 15 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI TbRAB18, a developmentally regulated Golgi GTPase from Trypanosoma brucei
SO Molecular and Biochemical Parasitology (2002), 121(1), 63-74
CODEN: MBIPDP; ISSN: 0166-6851
AB The trypanosomal secretory system is broadly similar to that of higher eukaryotes as proteins enter the system via the endoplasmic reticulum and are transported to the Golgi complex for elaboration of glycan chains. Importantly N-glycan processing is stage specific with only the bloodstream form (BSF) processing beyond the oligomannose form. Increased complexity of the BSF Golgi apparatus, as evidenced by morphol. studies, may underpin this higher activity, but few trypanosome-specific Golgi proteins have been described that may play a role in this developmental alteration. Here we describe a novel member of the T. brucei Rab family, TbRAB18, which is stage-regulated and highly expressed in the BSF while barely detectable in the insect stage. This stage-specific expression suggests the presence of a TbRAB18-dependent transport pathway required for survival in the mammalian host. Furthermore, data indicate that TbRAB18 localizes to membranes in close juxtaposition to structures stained with **BODIPY**-ceramide, a Golgi marker. Wild type TbRAB18, ectopically expressed in insect stage cells colocalizes with TbRAB31, and hence is targeted to the Golgi complex, consistent with the location of the endogenous protein in the bloodstream form, while **GTP** and

GDP-locked mutant isoforms demonstrate distinct localizations, suggesting that Golgi-targetting of TbRAB18 is nucleotide-state dependent. We also find that ectopic expression of TbRAB18 wild type and mutant isoforms has no detectable effect on the synthetic anteriograde trafficking probe, TbBiPN. Finally, the location, and hence function, of TbRAB18 are distinct from the closest metazoan homolog, murine Rab18; the latter protein is involved in endocytic transport pathways while clearly TbRAB18 is not. Our data indicate further complexity in the evolution of small GTPases, and highlight the need for robust functional data prior to assignment of members of complex gene families.

L15 ANSWER 16 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Golgi apparatus dynamics during mouse oocyte in vitro maturation: effect of the membrane trafficking inhibitor brefeldin A
SO Biology of Reproduction (2002), 66(5), 1259-1266
CODEN: BIREBV; ISSN: 0006-3363
AB The authors have studied Golgi apparatus dynamics during mouse oocyte in vitro maturation, employing both live imaging with the fluorescent lipid **BODIPY**-ceramide and immunocytochem. using several specific markers (β-COP, giantin, and TGN38). In germinal vesicle oocytes the Golgi consisted of a series of structures, possibly cisternal stacks, dispersed in the ooplasm, but slightly more concentrated in the interior than at the cortex. A similar pattern was detected in rhesus monkey germinal vesicle oocytes. These "mini-Golgis" were functionally active because they were reversibly disrupted by the membrane trafficking inhibitor brefeldin A. However, the drug had no visible effect if the oocytes had been previously microinjected with **GTP**-γ-S. During in vitro maturation the large Golgi apparatus structures fragmented at germinal vesicle breakdown, and dispersed homogenously throughout the ooplasm, remaining in a fragmented state in metaphase-II oocytes. Similarly to what has been reported using protein synthesis inhibitors, the presence of brefeldin A blocked maturation at the germinal vesicle breakdown stage before the assembly of the metaphase-I spindle. These results suggest that progression of murine oocyte maturation may require functional membrane trafficking.

L15 ANSWER 17 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Acyclic and dideoxy terminator preferences denote divergent sugar recognition by archaeon and Taq DNA polymerases
SO Nucleic Acids Research (2002), 30(2), 605-613
CODEN: NARHAD; ISSN: 0305-1048
AB The nucleotide-binding site in a variety of DNA polymerases was probed by analyzing incorporation of dideoxy and acyclic chain terminators. Family B archaeon DNA polymerases Vent, Deep Vent, 9°N and Pfu incorporated acyclic in preference to dideoxy terminators, while the Family A DNA polymerases Taq and Klenow preferred dideoxy terminators. These divergent biases suggest that significant differences exist in sugar recognition in these two classes of polymerases. Mutants of Vent (A488L) and Taq (F667Y) that increase incorporation of dideoxy terminators maintained the acyclic/dideoxy bias of the parent enzyme, while more efficiently incorporating both dideoxy and acyclic terminators. The preference of archaeon DNA polymerases for acyclic analogs was exploited in chain terminator DNA sequence and genotype anal. This technol. was addnl. aided by identification of specific dye-modified bases that improve terminator incorporation over that of the unmodified terminator. These three enhancing effects, (i) acyclic analogs, (ii) archaeon variants and (iii) specific dyes, appear to act additively and independently to increase terminator incorporation efficiency, collectively enhancing incorporation ~8000-fold over the wild-type incorporation of dideoxynucleotides. Fluorescent chain terminator DNA sequence traces demonstrate the applicability of these advances in improving terminator incorporation, as required in DNA sequence and genotype detns.

L15 ANSWER 18 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Labeled peptides, proteins and antibodies and processes and intermediates useful for their preparation
SO PCT Int. Appl., 158 pp.
CODEN: PIXXD2

AB The invention provides peptide synthons having protected functional groups for attachment of desired moieties (e.g. functional mols. or probes). Also provided are peptide conjugates prepared from such synthons, and synthon and conjugate preparation methods including procedures for identifying optimum probe attachment sites. Biosensors are provided having functional mols. that can locate and bind to specific biomols. within living cells. Biosensors can detect chemical and physiol. changes in those biomols. as living cells are moving, metabolizing and reacting to its environment. Methods are included for detecting **GTP** activation of a Rho GTPase protein using polypeptide biosensors. When the biosensor binds **GTP**-activated Rho GTPase protein, an environmentally sensitive dye emits a signal of a different lifetime, intensity or wavelength than when not bound. New fluorophores whose fluorescence responds to environmental changes are also provided that have improved detection and attachment properties, and that can be used in living cells, or in vitro.

L15 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain
SO Journal of Biological Chemistry (2001), 276(36), 33369-33374
CODEN: JBCHA3; ISSN: 0021-9258
AB Protection of heart against ischemia-reperfusion injury by ischemic preconditioning and KATP channel openers is known to involve the mitochondrial ATP-sensitive K+ channel (mitoKATP). Brain is also protected by ischemic preconditioning and KATP channel openers, and it has been suggested that mitoKATP may also play a key role in brain protection. However, it is not known whether mitoKATP exists in brain mitochondria, and, if so, whether its properties are similar to or different from those of heart mitoKATP. We report partial purification and reconstitution of a new mitoKATP from rat brain mitochondria. We measured K+ flux in proteoliposomes and found that brain mitoKATP is regulated by the same ligands as those that regulate mitoKATP from heart and liver. We also examined the effects of opening and closing mitoKATP on brain mitochondrial respiration, and we estimated the amount of mitoKATP by means of green fluorescence probe **BODIPY**-FL-glyburide labeling of the sulfonylurea receptor of mitoKATP from brain and liver. Three independent methods indicate that brain mitochondria contain six to seven times more mitoKATP per mg of mitochondrial protein than liver or heart.

L15 ANSWER 20 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Genotyping methods for determining single nucleotide variations and its diagnostic application
SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2
AB The present invention provides methods and kits for determining the identity of a nucleotide at a variant site on a target nucleic acid. The methods begin with the template-dependent amplification of a target sequence under defined conditions to achieve selective incorporation of a nucleotide analog at the variant site. Amplification product is then subjected to limited degradation to create products having allele-specific sizes, which are subsequently separated on the basis of size. Finally, the number of products and their sizes is to assessed to determine the identity of the nucleotide(s) at the variant site and the genotype of the organism from which the target was obtained. The present invention is exemplified by detecting an A/G polymorphism wherein the PCR extension reaction is conducted using the nucleotide derivative (α-SdATP) and the standard nucleotides dTTP, dCTP and **GTP**.

=> d ti, so, abs 21-29 L15

L15 ANSWER 21 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Fluorescent **BODIPY-GTP** analogs: Real-time measurement of nucleotide binding to G proteins
SO Analytical Biochemistry (2001), 291(1), 109-117
CODEN: ANBCA2; ISSN: 0003-2697
AB Three **BODIPY GTP**γS analogs (FL, 515, and TR), **BODIPY** FL GppNHp and **BODIPY** FL **GTP** mols. were

synthesized as possible fluorescent probes to study guanine nucleotide binding spectroscopically. Binding to Gαo increases baseline analog fluorescence by 6-, 8.5-, 2.8-, 3.5-, and 3.0-fold, resp. Binding of **GTPγS** and GppNHp analogs to Gαo is of high affinity (KD 11, 17, 55, and 110 nM, resp.) and reaches a stable plateau while fluorescence of **BODIPY** FL **GTP** shows a transient increase which returns to baseline. Furthermore, **BODIPY** FL **GTPγS** shows varying affinities for αo, αs, αi1, and αi2 (6, 58, 150, and 300 nM). The affinities of **BODIPY** FL GppNHp for all four Gα subunits are 10-fold lower than for **BODIPY** FL **GTPγS**. Half-times for the fluorescence increase are consistent with known GDP release rates for those proteins. Enhancement of fluorescence upon binding the Gα subunit is most likely due to a rotation around the γ-thiol (**GTPγS**) or the 3' ribose-hydroxyl (GppNHp) bond to relieve the quenching of **BODIPY** fluorescence by the guanine base. Binding to Gα exposes the **BODIPY** moiety to the external environment, as seen by an increase in sodium iodide quenching. The visible excitation and emission spectra and high fluorescence levels of these probes permit robust real-time detection of nucleotide binding. (c) 2001 Academic Press.

L15 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Infection of human endothelial cells with Bartonella bacilliformis is dependent on Rho and results in activation of Rho
SO Infection and Immunity (2000), 68(10), 5960-5969
CODEN: INFIBR; ISSN: 0019-9567
AB Bartonella bacilliformis was continuously internalized into human endothelial cells beginning shortly after addition of the bacteria and continuing for at least 24 h after infection in vitro, with a major increase in uptake occurring between 16 and 24 h. Preincubation of endothelial cells with C3 exoenzyme, which inactivated intracellular Rho-GTPase, blocked internalization of the bacteria. Addition of C3 exoenzyme at any time after addition of the bacteria blocked further internalization of bacteria, including the major uptake of bacteria internalized at 16 to 24 h. Rho, a key signaling protein in pathways involving actin organization, was directly shown to be activated in endothelial cells undergoing infection with B. bacilliformis, with maximal activation and translocation to the plasma membrane at 12 to 16 h. At late times of infection, most of the bacteria were found in a perinuclear location. Staining of the Golgi complex with specific markers, anti-human Golgin-97, anti-KDEL receptor, and **BODIPY**-TR ceramide, showed colocalization of bacteria in the Golgi complex region. Disruption of the Golgi complex with brefeldin A scattered the bacteria from this perinuclear location and resulted in inhibition of internalization of the bacteria in endothelial cells.

L15 ANSWER 23 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Method of sequencing nucleic acids by applying a computer alignment algorithm to electrophoretic separation patterns of dideoxy-terminated fragment mixtures
SO U.S., 29 pp., Cont.-in-part of U.S. 636,414, abandoned.
CODEN: USXXAM
AB The present invention describes a method of sequencing nucleic acids in which mixts. of oligonucleotide fragments are derived from sequencing reactions using combinations of the 2',3'-dideoxynucleoside 5'-triphosphate or 3' deoxynucleoside 5'-triphosphate terminators and appropriate concns. of four dNTPs (2'-deoxynucleoside 5' triphosphates, e.g., dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-**GTP**). These fragments are generated by enzymic extension of a primer hybridized to the single-stranded template DNA to be sequenced. In contrast to common slab gel sequencing methods, the method of the instant invention does not require precise alignment of the four separation sets of the terminated fragments to permit deduction of the DNA sequence. Instead the relative positions of the nucleotide bases in sep. mixts. can be deduced from binary-coded sequence string sets corresponding to the presence or absence of particular fragments by applying a computer alignment algorithm. In addition, the method possesses inherent redundancy in the sepns., which

facilitates sequence assignment by resolving sequence uncertainties or anomalies. The method was applied to the determination of the known sequence of M13mp18 DNA, and a lengthy stretch of sequence in the middle was correctly determined

L15 ANSWER 24 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Fhit-nucleotide specificity probed with novel fluorescent and fluorogenic substrates
SO Journal of Biological Chemistry (2000), 275(7), 4555-4560
CODEN: JBCHA3; ISSN: 0021-9258
AB Fhit, a member of the histidine triad superfamily of nucleotide-binding proteins, binds and cleaves diadenosine polyphosphates and functions as a tumor suppressor in human epithelial cancers. Function of Fhit in tumor suppression does not require diadenosine polyphosphate cleavage but correlates with the ability to form substrate complexes. As diadenosine polyphosphates are at lower cellular concns. than mononucleotides, we sought to quantify interactions between Fhit and competitive inhibitors with the use of diadenosine polyphosphate analogs containing fluorophores in place of one nucleoside. First generation fluorescent Fhit substrates were prepared in high yield by reacting thiol-reactive fluorescent dyes (7-diethylamino-4-methyl-3-(4'-maleimidylphenyl)-4-methylcoumarin and N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacine-3-yl)methyl)iodoacetamide with ATPγS tetralithium salts to yield ApppAMC and ApppBODIPY, resp. A second generation Fhit fluorescent substrate was prepared by conjugating N-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacine-3-yl)methyl)iodoacetamide to **GTP**γS tetralithium salt, yielding GpppBODIPY. These three compds. are effective Fhit substrates, producing AMP or GMP plus fluorophore diphosphates. GpppBODIPY cleavage is accompanied by a 5.4-fold increase in fluorescence because **BODIPY** fluorescence is quenched by stacking with guanine. Titration of unlabeled diadenosine polyphosphates, inorg. pyrophosphate, mononucleotides, and inorg. phosphate into fluorescent assays provided values of Km and KI as competitive inhibitors. The data indicate that Fhit discriminates between good substrates via kcat and against cellular competitors in equilibrium binding terms. Surprisingly, pyrophosphate competes better than purine mononucleotides.

L15 ANSWER 25 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Cell-cycle and developmental regulation of TbRAB31 localization, a **GTP**-locked Rab protein from Trypanosoma brucei
SO Molecular and Biochemical Parasitology (2000), 106(1), 21-35
CODEN: MBIPDP; ISSN: 0166-6851
AB Rab proteins are small GTPases that control the direction and timing of vesicle fusion during intracellular trafficking between membraneous compartments. Genome sequencing and EST anal. of Trypanosoma brucei indicates that the trypanosome Rab (TbRAB) gene family, and hence complexity of intracellular transport pathways, is intermediate between Saccharomyces cerevisiae and mammals. TbRAB31 is a constitutively expressed T. brucei Rab protein (formerly Trab7p) and is the product of one of two closely linked TbRAB genes, the other being TbRAB2 (TbRab2p, in: Field H, Ali BRS, Sherwin T, Gull K, Croft SL, Field MC. TbRab2p, a marker for the endoplasmic reticulum of Trypanosoma brucei, localizes to the ERGIC in mammalian cells. J. Cell Sci., 1999; 112:147-156), involved in EF to Golgi transport. TbRAB31 has high homol. to members of the Sec4/Ypt1 subfamily of Rab proteins from Saccharomyces cerevisiae and to Rab13 and Rab11 from higher eukaryotes. Recombinant TbRAB31 binds **GTP** but, unusually for a Rab protein, has undetectable GTPase activity resulting in a constitutively **GTP**-bound protein. Antibodies against TbRAB31 recognize a discrete structure located between the kinetoplast and nucleus in interphase procyclic cells; by contrast the structure is morphol. more complex in bloodstream form (BSF) parasites, consisting of at least two foci. TbRAB31 behavior was also studied during the cell cycle; TbRAB31 always localized to a discrete structure that duplicated very early in mitosis and relocated to daughter cells in a coordinate manner with the basal body and kinetoplast, suggesting the involvement of microtubules. Addnl. evidence suggests that TbRAB31 localizes to the trypanosome Golgi complex. Firstly, the interphase position of TbRAB31 is consistent with a Golgi location. Secondly, the

TbRAB31 structure is also recognized by cross-reacting antibodies to mammalian β-coatomer protein (β-COP), which localizes to the Golgi in mammalian cells. Thirdly, the fluorescent ceramide analog, **BODIPY**-TR-ceramide, a reliable marker of the mammalian Golgi apparatus, exhibited overlapping distribution with TbRAB31. The location of **BODIPY**-TR-ceramide was confirmed at the trypanosome Golgi by histochem. with diaminobenzidine and electron microscopy.

L15 ANSWER 26 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI A requirement for ARF6 in Fcγ receptor-mediated phagocytosis in macrophages
SO Journal of Biological Chemistry (1998), 273(32), 19977-19981
CODEN: JBCHA3; ISSN: 0021-9258
AB Phagocytosis requires extension of F-actin-rich pseudopods and is accompanied by membrane fusion events. Members of the ARF family of GTPases are essential for many aspects of membrane trafficking. To test a role for this family of proteins in Fcγ receptor-mediated phagocytosis, the authors utilized the fungal metabolite brefeldin A (BFA). The addition of 100 μM BFA to a subclone of RAW 264.7 macrophages disrupted the appearance and function of the Golgi apparatus as indicated by altered immunofluorescent distribution of β-COP and reduced efflux of **BODIPY** C5-ceramide, a phospholipid that normally accumulates in the Golgi apparatus In contrast, BFA had no effect on phagocytosis of IgG-coated erythrocytes. These results suggested that activation of BFA-sensitive ARFs is not required for phagocytosis. ARF6 is unique among members of the ARF family in that its membrane association is unaffected by BFA. Expression of ARF6 mutants defective in either **GTP** hydrolysis (Q67L) or binding (T27N) inhibited phagocytosis of IgG-coated erythrocytes and attenuated the focal accumulation of F-actin beneath the test particles. These results indicate a requirement for ARF6 in Fcγ receptor-mediated phagocytosis and suggest that ARF6 is an important mediator of cytoskeletal alterations after Fcγ receptor activation.

L15 ANSWER 27 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Cell sorting with fluorescent peptides
SO PCT Int. Appl., 58 pp.
CODEN: PIXXD2
AB A method for sorting cells included in a cell population is described. The method includes the step of first exposing the cell population to a biol. active fluorescent peptide containing peptide and light-emitting moieties. A first group of cells in the cell population are then labeled when the peptide of the biol. active fluorescent peptide binds to a corresponding receptor contained on (or in) each cell in the first group of cells. The first group of cells or a group of cells excluding the first group of cells are then sorted from the cell population.

L15 ANSWER 28 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI Real-time fluorescence measurement of cell-free endosome fusion: regulation by second messengers
SO Biophysical Journal (1996), 71(1), 487-494
CODEN: BIOJAU; ISSN: 0006-3495
AB A quant. real-time assay of cell-free endosomal vesicle fusion was developed and applied to study fusion mechanisms in endosomes from baby hamster kidney (BHK-21) cells. The assay is based on an irreversible .apprx.10-fold increase in **BODIPY**-avidin fluorescence on binding of biotinylated conjugates. **BODIPY**-avidin and biotin-dextran were internalized for 10 min at 37° into sep. populations of BHK-21 cells, and endosome fractions were prepared Postnuclear supernatant fractions underwent ATP- and temperature-dependent fusion, as measured in a sensitive custom-built microfluorometer by the continuous increase in **BODIPY**-avidin fluorescence. Fusion processes of efficiency >2.5% could be detected with 200-ms time resolution in sample vols. of 50 μL containing endosomes derived from .apprx.4 + 104 cells. The fusion time course consisted of a distinct lag phase (up to 10 min) in which little fusion occurred, followed by an approx. exponential rise (t1/2 10-30 min; fusion efficiency .apprx.15%). The lag phase was reduced by preincubation of sep. endosome fractions with ATP at 37° and by coincubation of

endosomes at 22° before the assay, suggesting a rate-limiting step involving binding of a soluble protein to the endosome membrane. Endosome fusion was strongly inhibited by **GTP**γS, N-ethylmaleimide, and AlF4-. Endosome fusion was not affected by phorbol myristate acetate but was significantly inhibited by cAMP and bovine brain calmodulin. The results establish a sensitive real-time fluorescence assay to quantify the kinetics and extent of endosome fusion in a cell-free system and demonstrate regulation of early endosome fusion by cytosolic second messengers.

L15 ANSWER 29 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
TI A deficiency of the small GTPase rab8 inhibits membrane traffic in developing neurons
SO Molecular and Cellular Biology (1995), 15(2), 918-24
CODEN: MCEBD4; ISSN: 0270-7306
AB One of the major activities of developing neurons is the transport of new membrane to the growing axon. Candidates for playing a key role in the regulation of this intense traffic are the small **GTP**-binding proteins of the rab family. The authors have used hippocampal neurons in culture and analyzed membrane traffic activity after suppressing the expression of the small **GTP**-binding protein rab8. Inhibition of protein expression was accomplished by using sequence=specific antisense oligonucleotides. While rab8 depletion resulted in the blockage of morphol. maturation in 95% of the neurons, suppression of expression of another rab protein, rab3a, had no effect, and all neurons developed normal axons and dendrites. The impairment of neuronal maturation by rab8 antisense treatment was due to inhibition of membrane traffic. Thus, by using video-enhanced differential interference contrast microscopy, the authors observed in the rab8-depleted cells a dramatic reduction in the number of vesicles undergoing anterograde transport. Moreover, by incubating antisense-treated neurons with **Bodipy**-labeled ceramide, a fluorescent marker for newly formed exocytic vesicles, the authors observed fluorescence labeling restricted to the Golgi apparatus, whereas in control cells labeling was found also in the neurites. These results show the role of the small GTPase rab8 in membrane traffic during neuronal process outgrowth.

=> d all 4, 13 L15

L15 ANSWER 4 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2004:995710 CAPLUS
DN 141:421720
ED Entered STN: 19 Nov 2004
TI High throughput screening fluorescent assay for guanylyl and adenylyl cyclase
IN Sunahara, Roger K.; Neubig, Richard R.
PA The Regents of the University of Michigan, USA
SO U.S. Pat. Appl. Publ., 14 pp.
CODEN: USXXCO
DT Patent
LA English
IC ICM C12Q001-68
ICS C12N009-22
NCL 435006000; 435199000
CC 7-1 (Enzymes)
Section cross-reference(s): 1
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2004229251	A1	20041118	US 2004-779404	20040213
PRAI US 2003-447074P	P	20030213		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2004229251	ICM	C12Q001-68
	ICS	C12N009-22
	NCL	435006000; 435199000

US 2004229251 ECLA C12N009/88

AB The present invention relates to methods of assaying nucleotide cyclase activity. In particular, the present invention relates to fluorescence-based methods of assaying guanylyl and adenylyl cyclase activity using fluorescently labeled **GTPγS**. The assay can be used for high throughput screening of both activators and inhibitors of cyclase.

ST nucleotide guanylyl adenylyl cyclase detn fluorescent assay

IT Animal cell line
(SF9, membrane-bound adenylyl cyclase type I determination in; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT Membrane, biological
(bilayer, nucleotide cyclase bound to; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT High throughput screening
(drug; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT Fluorescence
Fluorescent indicators
Fluorometry
High throughput screening
Test kits
(high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT Drug screening
(high throughput; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT Orphan receptors
RL: ANT (Analyte); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(nucleotide cyclase; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT 594840-72-9, **BODIPY FL-GTPγS**
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(fluorescently labeled substrate; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT 7439-96-5, Manganese, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(guanylyl cyclase activity in presence of; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT 9054-75-5, Guanylyl cyclase
RL: ANT (Analyte); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT 86-01-1, 5'-**GTP**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(recognition of **GTP** by adenylyl cyclase; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT 37589-80-3D, fluorescently labeled
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(substrate; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

IT 9012-42-4, Adenylyl cyclase
RL: ANT (Analyte); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(type II, VC1-ACGC mutant; high throughput screening fluorescent assay for guanylyl and adenylyl cyclase)

L15 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:540191 CAPLUS
DN 137:106001
ED Entered STN: 19 Jul 2002
TI Arrays of biological membranes and methods and use thereof
IN Fang, Ye; Frutos, Anthony G.; Jonas, Steven J.; Kalal, Peter J.; Lahiri, Joydeep
PA USA
SO U.S. Pat. Appl. Publ., 35 pp., Cont.-in-part of U.S. Ser. No. 854,786.

CODEN: USXXCO
DT Patent
LA English
IC ICM G01N033-53
ICS G01N033-542; C12M001-34
NCL 435007900
CC 9-1 (Biochemical Methods)
Section cross-reference(s): 7
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002094544	A1	20020718	US 2001-974415	20011009
	US 2002019015	A1	20020214	US 2001-854786	20010514
	WO 2002092833	A2	20021121	WO 2002-US11332	20020403
	WO 2002092833	A3	20031009		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	EP 1388010	A2	20040211	EP 2002-728731	20020403
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI	US 2000-224135P	P	20000810		
	US 2001-854786	A2	20010514		
	US 2001-974415	A	20011009		
	WO 2002-US11332	W	20020403		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2002094544	ICM	G01N033-53
	ICS	G01N033-542; C12M001-34
	NCL	435007900
US 2002094544	ECLA	G01N033/543K2B; G01N033/566
US 2002019015	ECLA	G01N033/543K2B; G01N033/566

AB The present invention overcomes the problems and disadvantages associated with prior art arrays by providing an array comprising a plurality of biol. membrane microspots associated with a surface of a substrate that can be produced, used and stored, not in an aqueous environment, but in an environment exposed to air under ambient or controlled humidities. Preferably, the biol. membrane microspots comprise a membrane bound protein. Most preferably, the membrane bound protein is a G-protein coupled receptor, an ion channel, a receptor serine/threonine kinase or a receptor tyrosine kinase.

ST G protein coupled receptor membrane array

IT Dopamine receptors
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DEV (Device component use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(D1; arrays of biol. membranes and methods and use thereof)

IT Dopamine receptors
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DEV (Device component use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(D1A; arrays of biol. membranes and methods and use thereof)

IT Fluorometry
(FRAP (fluorescence recovery after photobleaching); arrays of biol. membranes and methods and use thereof)

IT Neurotensin receptors
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DEV (Device component use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(NTR1; arrays of biol. membranes and methods and use thereof)

IT Silanes
RL: DEV (Device component use); USES (Uses)

(amino; arrays of biol. membranes and methods and use thereof)
IT Adsorption
Affinity
Amino group
Carboxyl group
Coating materials
Contact angle
Hydroxyl group
Immobilization, molecular or cellular
Linking agents
Mass spectrometry
Membrane, biological
Microarray technology
Microtiter plates
Molecular association
Molecular orientation
Molecular surface
Phosphate group
Protein microarray technology
Radiography
Sample preparation
Self-assembled monolayers
(arrays of biol. membranes and methods and use thereof)
IT G protein-coupled receptors
Ion channel
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(arrays of biol. membranes and methods and use thereof)
IT Acid halides
Esters, uses
Glass, uses
Metals, uses
Phosphatidylcholines, uses
Plastics, uses
Polymers, uses
Silanes
Thiols (organic), uses
RL: DEV (Device component use); USES (Uses)
(arrays of biol. membranes and methods and use thereof)
IT Imaging
(chemiluminescence; arrays of biol. membranes and methods and use thereof)
IT Imaging
(fluorescent; arrays of biol. membranes and methods and use thereof)
IT Functional groups
(hydrazino group; arrays of biol. membranes and methods and use thereof)
IT Functional groups
(isocyanato group; arrays of biol. membranes and methods and use thereof)
IT Functional groups
(maleimide; arrays of biol. membranes and methods and use thereof)
IT Proteins
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(membrane; arrays of biol. membranes and methods and use thereof)
IT Surface plasmon
(resonance imaging; arrays of biol. membranes and methods and use thereof)
IT Light scattering
(resonance; arrays of biol. membranes and methods and use thereof)
IT Acids, uses
Acrylic polymers, uses
Alcohols, uses
Amines, uses
Anhydrides
RL: DEV (Device component use); USES (Uses)
(silyl; arrays of biol. membranes and methods and use thereof)

IT Microscopes
(slides; arrays of biol. membranes and methods and use thereof)
IT Functional groups
(sulfonate group; arrays of biol. membranes and methods and use thereof)
IT Alcohols, uses
Amines, uses
Halogen compounds
RL: DEV (Device component use); USES (Uses)
(thioalkyl; arrays of biol. membranes and methods and use thereof)
IT Agglutinins and Lectins
RL: ARU (Analytical role, unclassified); PEP (Physical, engineering or chemical process); PYP (Physical process); ANST (Analytical study); PROC (Process)
(wheat germ; arrays of biol. membranes and methods and use thereof)
IT Adrenoceptors
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); DEV (Device component use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(β1; arrays of biol. membranes and methods and use thereof)
IT Opioid receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(δ-opioid; arrays of biol. membranes and methods and use thereof)
IT Opioid receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(μ-opioid; arrays of biol. membranes and methods and use thereof)
IT 228265-94-9, **BODIPY** FL-Sch 23390
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**BODIPY** FL-Sch 23390; arrays of biol. membranes and methods and use thereof)
IT 287384-28-5D, **BODIPY** TMR, conjugates with neurotensin and CGP 12177
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**BODIPY** TMR; arrays of biol. membranes and methods and use thereof)
IT 86-01-1, **GTP** 37589-80-3
RL: ANT (Analyte); ANST (Analytical study)
(arrays of biol. membranes and methods and use thereof)
IT 340830-03-7, Receptor tyrosine kinase
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(arrays of biol. membranes and methods and use thereof)
IT 60-00-4, EDTA, analysis 139-13-9, Nitrilotriacetic acid 63741-19-5 265310-03-0
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(arrays of biol. membranes and methods and use thereof)
IT 9054-75-5, Guanylate cyclase 71310-21-9, 11-Mercaptoundecanoic acid 73768-94-2, 11-Mercaptoundecanol 114896-32-1, 16-Mercaptohexadecanol 155638-19-0, 11-Mercaptoundecylamine 155734-90-0 187099-99-6, Texas Red DHPE 340700-49-4, Receptor serine-threonine protein kinase
RL: ARU (Analytical role, unclassified); DEV (Device component use); PEP (Physical, engineering or chemical process); PYP (Physical process); ANST (Analytical study); PROC (Process); USES (Uses)
(arrays of biol. membranes and methods and use thereof)
IT 39379-15-2, Neurotensin 39379-15-2D, Neurotensin, conjugates with **BODIPY** TMR 62229-50-9, Epidermal growth factor 81047-99-6D, CGP 12177, conjugates with **BODIPY** TMR
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(arrays of biol. membranes and methods and use thereof)
IT 63-89-8, L-α-Dipalmitoylphosphatidylcholine 6382-82-7, γ-Aminopropylsilane 7440-57-5, Gold, uses 18194-24-6, L-α-Dimyristoylphosphatidylcholine 18194-25-7, 1,2-Dilauroyl-sn-glycero-3-phosphocholine 69839-68-5, 16-Mercaptohexadecanoic acid
RL: DEV (Device component use); USES (Uses)
(arrays of biol. membranes and methods and use thereof)
IT 119418-04-1, Galanin
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(receptor; arrays of biol. membranes and methods and use thereof)